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LETTERS

edited by Etta Kavanagh

Retraction of an Interpretation

IN THE REPORT "STRUCTURE OF THE 8200-YEAR COLD EVENT REVEALED BY A SPELEOTHEM trace element record" (1), we presented a 7762- μm -long ion probe trace element traverse chosen to include the 8200-year event as detected in a previously published laser ablation oxygen isotope study from the same stalagmite (2). The oxygen isotope anomaly was distinct and dropped 8‰ below baseline values to a low value for the entire Holocene of -12‰ and was reproducible on a reverse track. However, recent reanalysis of the calcite believed to contain the oxygen isotope anomaly suggests that the anomaly was probably an analytical artifact possibly caused by laser ablation-induced fracturing during the original analysis (3). Consequently, without the original $\delta^{18}\text{O}$ "marker," the precise location in the stalagmite of calcite deposited during the 8200-year event is uncertain.

The trace element data in this Report, previously believed to correspond precisely with the entire 8200-year event, are now believed to represent the hydrological and bioproductivity response in western Ireland to a cold/dry event of uncertain provenance and intensity. The U-Th-derived dates of the event correspond approximately with the 8200-year event in Greenland ice cores, but without the additional guidance of the $\delta^{18}\text{O}$ anomaly, the precise timing in relation to the 8200-year event is now somewhat ambiguous. Unfortunately, it is now unlikely that the approximately 114-year duration ion probe track coincides with the entire 8200-year event (if at all); thus, the ~ 37 -year estimate derived for its duration is probably no longer accurate. However, the trace element data remain robust and are interpreted as reflecting colder and drier conditions in western Ireland, followed by the return to more maritime conditions at the end of the first-order trace element anomaly. Additionally, the novel application of annual trace element cycles to build a high-resolution chronology and reconstruct paleoseasonality remains unchanged.

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The Dangers of Advocacy in Science

IN MATTERS OF POLICY, MANY SCIENTISTS view "advocacy" as a dangerous word. In peer-reviewed literature, many scientists practice a subtler form of advocacy in pushing their methods, results, and conclusions. Call this IMRAD (Introduction, Methods, Results, and Discussion) advocacy: Once bold claims about a poorly tested method or

weak result are published, their sins are forgiven and they can be worked into future introductions and discussions at will. IMRAD advocates often stretch available data, gloss over uncertainties in their evidence, and ignore contrary results.

This occurs throughout the hierarchy of journals. One would hope that it would be least common in prestigious journals. On the other hand, top journals have limited space; they emphasize papers with broad, seemingly decisive conclusions but

passively encourage readers not to worry about methods (or rebuttals). Often, this form of advocacy is obvious only to the small percentage of any journal's readers that have scientific expertise in a specialized area—a small pool of appropriate reviewers (1).

As with policy advocacy, there is a gray area between objective justification and flagrant, half-supported promotion. Somewhere in the middle sits the honest, often acrimonious debate necessary for scientific progress. Would anyone disagree that pub-

"Would anyone disagree that publishing overly liberal conclusions is poor science...?"

—Gitzen

lishing overly liberal conclusions is poor science, that the personal rewards of doing so far outweigh risks, or that the peer-review process should strip papers of this garbage? Humiliation could assist rebuttals and time in the self-correcting process of science. For example, each professional society should survey members at year's end to decide on the five papers in their field with the most overly inflated claims. The authors, approving reviewers, and subject editors could receive suitable prizes.

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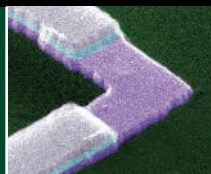
Controversy Over EmrE Structure

TWO X-RAY STRUCTURES OF EMRE, THE SMALLEST ion-coupled multidrug transporter, have provided a cautionary tale about the difficulty of determining the three-dimensional structures of membrane proteins and the dangers of ignoring biochemical results. The structures have since been retracted (1, 2), but the intriguing and controversial idea that the



Patterns in scientific
discoveries

761



Strumming a
nano tune

762

protomers in the EmrE homodimer adopt an antiparallel transmembrane orientation continues to have support. In their Report “Emulating membrane protein evolution by rational design” (2 March, p. 1282), M. Rapp *et al.* describe results that seem to support such an antiparallel arrangement and that are thought to provide the missing link in membrane protein evolution. However intriguing the results may be, the starting point is too fuzzy and ignores biochemical results. The interpretation is based on the assumption that EmrE displays a dual topology. However, the x-ray structures have been retracted, and the results that support such an assumption in Rapp *et al.* cannot be taken at face value, as the authors admit, because the large fusion proteins they used to determine the topology

of a protein two to four times smaller seem to bias the results. This is unfortunate, and it would be helpful to apply alternative approaches, which may be more time-consuming but less ambiguous.

In their Perspective “A missing link in membrane protein evolution” (2 Mar., p. 1229), B. Poolman *et al.* claim that our rigorous demonstration that EmrE with a parallel topology of the protomers is fully functional was based on the now obsolete structural model. On the contrary, our work showed that this model was incorrect, rather than being based on it (3).

There is suggestive evidence that some homologs of EmrE that function as heterodimers or a properly mutated EmrE display an antiparallel topology of the protomers

relative to each other. If the case can be made for antiparallel heterodimers, what makes it so different for a homodimer? If antiparallel homodimers exist, researchers would be faced with fascinating questions about the insertion and assembly of these proteins in the membrane (4). Do the protomers insert with a random topology and wait for the next randomly inserted one? To our knowledge, the existence of homodimers with an antiparallel orientation of the monomers has not yet been biochemically demonstrated.

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Response

IN RECENT MONTHS, EMRE HAS TAKEN CENTER stage in the world of membrane proteins because there are opposing views on its

membrane topology (1) and because two x-ray structures of EmrE were recently retracted (2, 3).

EmrE is an unusually intriguing protein. It is by far the most well-studied representative of the bacterial small multidrug-resistance (SMR) proteins, a family of potential drug targets, and it may be the first example of a "dual topology" protein, i.e., a homodimeric protein composed of two identical monomers with opposite orientations in the membrane (4).

The final proof for a dual topology for EmrE is still lacking. So, what is the evidence? First, the dual topology idea was originally proposed on the basis of an early electron crystallography structure (5, 6). This structure, albeit of rather low resolution, is still the gold standard, since the two-dimensional crystals bind substrate with nM affinity.

Second, a steadily increasing number of SMR proteins have been shown to be heterodimers composed of two homologous monomers [e.g., (7)]. In at least one case (the EmrE homologs YdgE/YdgF in *E. coli*), the two monomers have been shown to adopt opposite orientations in the membrane (8), and topology predictions suggest that this is the general rule for heteromeric SMR proteins (9). By extension, a dual topology for homodimeric EmrE seems likely.

Third, by mutating positively charged residues in the loops connecting the transmembrane helices, we have constructed two EmrE variants that insert with either N_{in} - C_{in} or N_{out} - C_{out} orientations. These variants are non-functional when expressed alone, but make cells resistant to ethidium bromide when co-expressed (10), as does wild-type EmrE. The complementation between the two oppositely oriented EmrE variants suggests that they form an antiparallel heterodimer, like other heteromeric SMR proteins.

On the other hand, the Schuldiner lab has reported that a chemically cross-linked EmrE dimer is active after reconstitution in vitro (11). With the cross-linked residues chosen such that they should not be able to form a

cross-link in an antiparallel dimer (according to the now retracted x-ray structure), this result provides an argument against a dual topology. But is this biochemical finding with solubilized, cross-linked protein compelling enough to override the structural, coexpression, and evolutionary arguments that support a dual topology for EmrE? We think not. In any case, given its current "15 minutes of fame" (12), EmrE will no doubt attract enough attention for the debate over its topology to be resolved in the normal scientific way: by more and better experiments.

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12. Warhol photo exhibition, Stockholm, 1968, in *Bartlett's Familiar Quotations*, J. Kaplan, J. Justin, Eds. (Little, Brown, New York, 1992).

Response

THE QUESTION OF THE MEMBRANE ORIENTATION of the two subunits in the multidrug efflux protein EmrE is befuddled by two separate issues. First, there are the x-ray crystallography studies of EmrE that were recently retracted (1, 2). Clearly, invalid

structural models cannot be used as a lead in any study. Second, there are biochemical data that lead to different conclusions on the subunit orientation of EmrE. Von Heijne and colleagues have provided evidence for an antiparallel orientation of the subunits (3), whereas Schuldiner and colleagues support a parallel orientation (4).

Conflicting models are proposed all the time in the process of scientific progress, and to choose which model is most probable, we have to scrutinize the available data and interpretations. Which studies are at hand? First, there is the only piece of structural information left: the 3D model of EmrE-based electron crystallography experiments (5), which provides a reliable structural model of EmrE. Unfortunately, the resolution is too low to reach definitive conclusions on the orientation of the subunits.

Second, there are the biochemical studies of the Schuldiner group (6–8). Soskine *et al.* (8) argue in favor of a parallel orientation of the subunits because their cross-linking data are inconsistent with the antiparallel orientation of the subunits observed in the now-obsolete x-ray crystallographic structural model. Both the design of their experiments (the positions of the engineered cysteines and the calculated intermolecular distances between the residues) and the interpretation of their data were based on the structural model that has since been shown to be incorrect (1). Moreover, the combination of high concentrations of detergent in the experiments, possibly leading to increased conformational flexibility of the protein, and the relatively large span of the applied cross-linker severely limit the validity of the approach. Consequently, we feel that the cross-linking data are not necessarily in conflict with an antiparallel orientation of the subunits (9).

CORRECTIONS AND CLARIFICATIONS

Reports: "Synthesis of ultra-incompressible superhard rhenium diboride at ambient pressure" by H.-Y. Chung *et al.* (20 April, p. 436). After publication, the original authors concluded that Robert W. Cumberland (Department of Chemistry and Biochemistry and the Department of Materials Science and Engineering, University of California, Los Angeles, CA 90024, USA; current address: HRL Laboratories, Malibu, CA 90265, USA), who was acknowledged, contributed sufficiently to the work to be listed as an author. This change was approved by the Vice Chancellor for Research at UCLA. The authors should now be:

Hsiu-Ying Chung,* Michelle B. Weinberger,* Jonathan B. Levine, Robert W. Cumberland, Abby Kavner, Jenn-Ming Yang, Sarah H. Tolbert, Richard B. Kaner

(*These authors contributed equally to this work).

Reports: "Causal reasoning in rats" by A. P. Blaisdell *et al.* (17 February 2006, p. 1020). There are three minor typos in the Supporting Online Materials. First, test sessions for Experiments 1, 2a, and 2b were 60 minutes. Second, the number of background nose pokes in Experiment 1 were 2793 ± 571 (Conditions Intervene-T), 3051 ± 991 (Condition Observe-T), 2885 ± 823 (Condition Intervene-N), and 2849 ± 514 (Condition Observe-N). Third, in Experiment 1, the *F* value for the planned comparison between condition Intervene-T versus Observe-T was 9.07, $p < 0.05$. A reanalysis of the data from Experiment 2b revealed that the test-trial data for one subject from group Unpaired-Observe was inadvertently counted twice in the statistical analyses. A reanalysis on the corrected data results in a change of three *F* values by a tenth of a point or less, and thus has no effect on the outcome of the analyses. The authors failed to revise the caption in the corrected Fig. 1 [*Science* **314**, 595 (2006)]. The *F* values were slightly different from those reported. Corrected values show the main effect of inference type = $F(1, 21) = 4.57$, $P < 0.05$, and the interaction = $F(1, 21) = 5.69$, $P < 0.05$.

Letters to the Editor

Letters (~300 words) discuss material published in *Science* in the previous 3 months or issues of general interest. They can be submitted through the Web (www.submit2science.org) or by regular mail (1200 New York Ave., NW, Washington, DC 20005, USA). Letters are not acknowledged upon receipt, nor are authors generally consulted before publication. Whether published in full or in part, letters are subject to editing for clarity and space.

Third, there are the studies of von Heijne and colleagues (3). Contrary to Schuldiner's suggestion, the interpretation of the data of Rapp *et al.* is not dependent on the prior assumption that EmrE displays a dual topology. The experiments were designed to discriminate between two alternative scenarios: parallel versus antiparallel orientation of the subunits. Schuldiner is correct in arguing that fusing a large reporter domain to a protein like EmrE may influence its orientation in the membrane. However, von Heijne and colleagues clearly recognize this point, and in a series of *in vivo* complementation studies with the EmrE protein subtly mutated to obtain a unique orientation, they showed that only an antiparallel arrangement of the EmrE subunits is functional. Schuldiner has proposed an alternative evolutionary model, but the scenario presented in (4) depends on many chance assumptions and is not very likely. The antiparallel topological model proposed by von Heijne and colleagues is also supported by the recent data of Zhang *et al.* (10).

We believe that both groups performed

solid experiments. Our view (9) is that the experiments from the Schuldiner group do not sufficiently discriminate between a dual topology model and a parallel model. Their evidence for parallel topology is based on cross-linking results with protein in the detergent-solubilized state, a nonnatural environment. In contrast, the von Heijne group used the native lipid membrane to study the function of EmrE. We therefore consider the dual topology model of EmrE more compelling—for now.

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A Clarification on Centrifugal Force

THE ARTICLE "SPINNING A NUCLEAR COME-back" (News Focus, 30 March, p. 1782) contains an erroneous statement about centrifugal force. The article states that "centrifugal forces pushed the gas outward, against the spinning wall." There is, in fact, no force pushing the gas outward. Instead, as covered in Newton's first and second laws, a force is required to prevent the gas from going in a straight line (and thereby accelerating the gas due to its constant change in direction). This force (termed centripetal force) acts inward toward the center of rotation and is provided by contact of the gas with the spinning wall.

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